Dow Petition (12-272-01p) for Determination of Non-regulated Status of DAS-81419-2 Soy

OECD Unique Identifier: DAS-81419-2

Plant Pest Risk Assessment

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A. Introduction

This plant pest risk assessment is to determine whether Dow AgroSciences LLC (DAS) event 81419-2 Insect Resistant Soybean (*Glycine max* cultivar Maverick) (hereafter DAS-81419-2) is unlikely to pose a plant pest risk. If the Animal and Plant Health Inspection Service of the United States Department of Agriculture (USDA-APHIS) determines that a genetically engineered (GE) organism is unlikely to pose a plant pest risk, APHIS then has no regulatory authority over that organism under 7 Code of Federal Regulations (CFR) part 340. APHIS administers the regulations 7 CFR part 340 under the authority of the Plant Protection Act of 2000[1].

Potential impacts in this plant pest risk assessment are those that pertain to plant pest risk associated with DAS-81419-2 and its progeny and their use in the absence of confinement. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if DAS-81419-2 is unlikely to pose a plant pest risk. USDA-APHIS has prepared this plant pest risk assessment in response to a petition (DAS 2012) from DAS. APHIS regulation 7 CFR part 340.6(c) stipulates the information needed for consideration in a petition for nonregulated status. APHIS evaluated information submitted by the applicant related to plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, potential impacts on non-target organisms, weediness of the regulated article, impacts on the weediness of plants with which it can interbreed, transfer of genetic information to organisms with which it cannot interbreed, and potential changes to agricultural or cultivation practices with respect to the likelihood of exacerbating plant disease or pests. An analysis of agricultural or cultivation practices associated with DAS-81419-2 and its potential impacts on the environment will be considered in the NEPA analysis. A thorough assessment of the effects of the determination on non-target organisms including beneficial organisms and threatened and endangered species will also be considered in the NEPA assessment. An environmental assessment on a similar insect resistant soybean with similar cultivation practices as the proposed DAS-81419-2 resulted in a FONSI (Monsanto 09-082-01p).

DAS-81419-2 expresses the insecticidal crystal (Cry) proteins Cry1Ac and Cry1F originally from the naturally-occurring soil bacterium, *Bacillus thuringiensis* (Bt). Cry1Ac and Cry1F provide protection against several lepidopteran pests of soybean, including soybean looper (*Chrysodeixis includens*), velvetbean caterpillar (*Anticarsia gemmatalis*), bean shoot borer (*Epinotia aporema*), fall armyworm (*Spodoptera frugiperda*), and tobacco budworm (*Heliothis virescens*). In addition, DAS-81419-2 expresses the phosphinothricin acetyltransferase (PAT) protein from the soil bacterium *Streptomyces viridochromogenes*. The PAT protein provides tolerance to the herbicide glufosinate and was used as a selectable marker during the development of

[1] Section 403 (14) of the Plant Protection Act (7USC Sec 7702(14)) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

DAS-81419-2. The transgenes for Cry1Ac, Cry1F and PAT expression were introduced into soybean via Agrobacterium-mediated transformation of soybean cotyledonary node explants. The disarmed *Agrobacterium tumefaciens* strain EHA101 (Hood et al., 1986), carrying the binary vector; pDAB9582, with the selectable marker PAT and the genes of interest: cry1Ac (synpro), and cry1Fv3 within the T-DNA region, were used to initiate transformation. Because three of the regulatory sequences used to facilitate expression of the insect resistance and herbicide tolerance genes in DAS-81419-2 were derived from plant pests (Cassava Vein Mosaic virus, *A.tumefaciens*, and *Streptomyces viridochromogenes*), DAS-81419-2 has been considered a regulated article under the APHIS regulations 7 CFR part 340.

B. Development of DAS-81419-2 Soy

DAS-81419-2 is currently only grown in the United States (U.S.) for breeding and seed increase activities due to the limited number of acres that are consistently exposed to susceptible pest insect pressure. This soybean is targeted for commercialization in Brazil and Argentina where insect pressure is consistently heavy, resulting in significant yield loss. On average, in the southern U.S. about one-third of the soybean acres in Georgia, Louisiana, and North Carolina have been sprayed regularly with insecticides since 1991 (Gianessi 2009). Insecticides are used on approximately 50% of the soybean acreage in Georgia for lepidopteran pests with velvetbean caterpillar being the most targeted pest (Gianessi et al., 2002). The velvetbean caterpillar occurs predominantly in Central and South America, but can overwinter in the southern U.S.in areas such as Florida and Southern Texas (Johnson et al., 1991). Although a major soybean pest in Central and South America, this caterpillar is only occasionally a pest of soybean production in Southeastern United states, although it cannot overwinter there (Johnson et al., 1991). Approximately 40% of the soybean acreage in Louisiana is treated with insecticides for lepidopteran pests, with soybean looper being the main target (Gianessi et al., 2002) Soybean looper overwinters in South Florida and South Texas within the U.S. and its distribution spreads down to Argentina and Chile in South America (Eichlin and Cunningham, 1978; Lafontaine and Poole, 1991). Chemical insecticides can have limited efficacy in controlling lepidopteran infestations in soybean. Narrow application windows, the emergence of insecticide resistance, and public pressure for reduced pesticide use limit the desirability of this approach to pest management for commercial growers (Thomas and Boethel, 1994). Resistance to pyrethroids is widespread across the southern U.S. (Felland et al., 1990; Leonard et al., 1990) and the soybean looper has already developed extensive insecticide resistance (Thomas and Boethel, 1994).

As mentioned earlier, DAS-81419-2 expresses two insecticidal proteins, Cry1Ac and Cry1F, originally from the naturally-occurring soil bacterium, *B.thuringiensis*. Cry1Ac and Cry1F provide plant protection against several target lepidopteran pests. In addition, DAS-81419-2 expresses the PAT protein from the soil bacterium *S. viridochromogenes*. The PAT protein provides tolerance to the herbicide glufosinate and was used as a selectable marker during the development of DAS-81419-2. The transgenes for Cry1Ac, Cry1F, and PAT expression were introduced into soybean via Agrobacterium-mediated transformation. Cry1Ac and Cry1F are expressed in the tissues of the soybean plants throughout the growing season, providing control of target lepidopteran pests of

soybeans. The two Cry proteins expressed; Cry1Ac and Cry1F modes of action differ with respect to receptor binding. Cry1Ac and Cry1F bind to different receptors in the midgut of the target soybean insect pest tobacco budworm (Jurat-Fuentes and Adang, 2001). Cry1Ac binds to at least three sets of receptors while Cry1F binds to at least two, only one of which also binds Cry1Ac. The major receptor for Cry1Ac is not recognized by Cry1F (Jurat-Fuentes and Adang, 2006). Such incomplete shared binding is expected to lead to incomplete cross-resistance when resistance is mediated by receptor changes. Bt gene pyramiding offered by DAS-81419-2 is expected to offer greater durability than Bt crops carrying a single Bt trait and provides protection against the development of insect resistance (DAS 2012, pg. 19).

There is a long history of safe use concerning crystal (Cry) proteins derived from *B.thuringiensis* (Ferre' and Van Rie, 2002). Bt corn and cotton expressing variations of Cry1Ac or Cry1F have been cultivated for commercial use in the U.S. and other countries for more than a decade. In 1997, the United States Environmental Protection Agency (EPA) established an exemption from the requirement of a tolerance for the plant-incorporated protectant Cry1Ac in all plants (40 CFR part 174.510). Later, EPA established an exemption from the requirement of a tolerance for the plant-incorporated protectant Cry1F in cotton (40 CFR part 174.504) and in corn (40 CFR part 174.520). DAS has filed a petition with EPA for an exemption from the requirement of a tolerance for Cry1F as expressed in soybean in 2012 (DAS 2012, pg. 20).

DAS expects to make submissions beginning 2012 to the regulatory authorities of other countries for import clearance and cultivation approval. Deregulation of DAS-81419-2 by APHIS is a prerequisite in achieving regulatory approvals in many of these countries (DAS 2012, pg. 20). DAS has submitted an application for a Federal Insecticide, Fungicide, and Rodenticide act (FIFRA) Section 3 seed increase registration with EPA to support future breeding and seed increase activities in the United States. Under the Section 3 seed increase registration, commercial sale of DAS-81419-2 in the U.S. is prohibited. At present, DAS does not plan to commercialize DAS-81419-2 in the U.S. due to the limited number of acres that consistently experience sufficient lepidopteron insect pressure (DAS 2012. pg. 4).

Description of the Genetic Modifications

The publicly available soybean cultivar "Maverick" was used as the recipient line for the generation of DAS-81419-2. DAS-81419-2 was generated using Agrobacterium-mediated transformation using plasmid pDAB9582. Transgenes in this soybean occurred as a single integration of the T-DNA insert from plasmid pDAB9582 containing two synthetic genes, *cry1Ac* (synpro) and *cry1Fv3*, obtained from *B. thuringiensis* as well as a pat gene from *S. viridochromogenes*. The full insert was stably integrated and inherited across breeding generations, and no plasmid backbone sequences are present in DAS-81419-2. The T-DNA insert in the plasmid contains a single, intact copy of each of the plant transcription units for the *cry1Fv3, cry1Ac* (synpro), and *pat* genes. In addition, a minor (<100 bp) fragment of the cry1Ac (synpro) gene was identified at the 5′ end of the T-DNA insert in a complementary orientation. Three gene expression cassettes are

present in the T-DNA region of plasmid pDAB9582 for insertion into soybean. (DAS 2012, pgs. 29-30).

Expression of Cry1Fv3 is controlled by the AtUbi10 promoter from *Arabidopsis thaliana* and the AtuORF23 3′UTR sequence from *A.tumefaciens* plasmid pTi15955. The AtUbi10 promoter is known to drive constitutive expression of the genes it controls (Norris et al., 1993). The amino acid sequence of the Cry1F protein is identical to that expressed in cotton event DAS-24236-5 which was deregulated by USDA APHIS in 2004 (USDA, 2004).

Expression of Cry1Ac (synpro) is controlled by the CsVMV promoter from Cassava Vein Mosaic virus and the AtuORF23 3′UTR sequence from *A. tumefaciens* plasmid pTi15955. The Cassava Vein Mosaic virus is a double stranded DNA virus which infects cassava plants (*Manihot esculenta* Crantz) and has been characterized as a plant pararetrovirus belonging to the caulimovirus subgroup. The CsVMV promoter is known to drive constitutive expression of the genes it controls (Verdaguer et al., 1996). The *cry1Ac* (synpro) gene sequence and the corresponding Cry1Ac amino acid sequence are identical to that expressed in cotton event DAS-21023-5 which was deregulated by USDA APHIS in 2004 (USDA, 2004).

Expression of the *pat* gene is controlled by the CsVMV promoter from Cassava Vein Mosaic virus and AtuORF1 3′ UTR sequence from *A.tumefaciens* plasmid pTi15955. The CsVMV promoter driving pat expression is the same as that driving expression of cry1Ac (synpro). The function of AtuORF1 (GenBank Accession: CAA25163) in pTi15955 (GenBank Accession: X00493) was not identified (Barker et al., 1983), but its translated amino acid sequence has a significant similarity with an indole-3-lactate synthase (GenBank Accession: AAK90967) from *A. tumefaciens* str. C58. The *pat* gene has been widely used both as a selectable marker and herbicide tolerance trait in previously deregulated products (USDA, 1996, 2001, 2004, 2005).

DAS provided evidence demonstrating that:

1) The transgene insert in DAS-81419-2 occurred as a single integration of the T-DNA insert from plasmid pDAB9582, including a single, intact copy of each of the plant transcription units for the *cry1Fv3*, *cry1Ac* (synpro), and *pat* genes. In addition, a minor (<100 bp) fragment of the *cry1Ac* (synpro) gene was identified at the 5′ end of the T-DNA insert. Southern blot analyses were prepared from leaf material from five breeding generations with the non-transgenic near isogenic variety Maverick as the control material, and plasmid DNA of pDAB9582 used as the positive control. Southern blot hybridization bands of the expected sizes were detected in all of the positive samples while none were detected in the negative control samples. The hybridization pattern was consistent across all five generations. (DAS 2012, Section 5.1-5.2, pgs. 40-62).

2) This data indicates that no plasmid backbone sequences from pDAB9582 have been integrated into DAS-81419-2 soybean (DAS 2012, Figs.26-28, pgs. 63-65).

3) The full insert was stably integrated and inherited across five breeding generations and the inserted DNA and resistance traits both segregate as expected (3:1) according to Mendel's Laws of Segregation as shown with data from a chi-square goodness of fit test across three populations of two generations (DAS 2012, pgs. 66-67).

C. Expression of the Gene Product and Changes to Plant Metabolism

USDA-APHIS assessed whether changes in plant metabolism or composition in DAS-81419-2 is likely to alter its plant pest risk. For this analysis DAS-81419-2 was compared to its non-transgenic near isogenic comparator (Maverick), as well as six non-transgenic lines (IL 3503, Porter 75148, DSR 75213-72, Pioneer 93M62, HiSOY 38C60, and Williams 82) conducted in 2011 at 10 sites located in Richland, Iowa; Atlantic, Iowa; Carlyle, Illinois; Wyoming, Illinois; Frankfort, Indiana; Fisk, Missouri; La Plata, Missouri; York, Nebraska; Brunswick, Nebraska; and Germansville, Pennsylvania (DAS 2012, pg. 81).

Summary of Cry1Ac, Cry1F, and PAT Characterization

The Cry1Ac protein expressed in DAS-81419-2 is a synthetic version of Cry1Ac1, from *B. thuringiensis* subsp. *kurstaki* strain HD73. The Cry1F protein expressed in DAS-81419-2 soybean is a synthetic version of Cry1F from *B. thuringiensis* subsp. *aizawai* strain PS81I. Biochemical characterization demonstrated that the DAS-81419-2 soybean-derived and *Pseudomonas*-derived Cry proteins used for analysis are equivalent thereby supporting the use of *Pseudomonas*-derived Cry proteins for safety assessment. The PAT protein was derived from *S. viridochromogenes*, a gram-positive soil bacterium. The PAT protein produced in DAS-81419-2 was shown to be substantially equivalent to that produced in *E. coli* which was used for protein analysis. Western blot analysis and lateral flow strip assays demonstrated that the PAT protein expressed in DAS-81419-2 had the expected molecular weight and immunoreactivity. Furthermore, the sequence of the PAT protein is identical to the PAT protein expressed in other deregulated transgenic crops such as "LibertyLink" soybean (USDA, 1996, 2001, 2004, 2005).

Expression of the Cry1Ac, Cry1F, and PAT Proteins in Plant Tissues

A field expression study was conducted at 10 locations in U.S. during 2011 (DAS 2012, Appendix 5, pgs. 188-190). Plant tissues sampled included leaf, grain, root, and forage. Leaf tissues were collected at the V5 and V10-12 stages, and root and forage were collected at the R3 stage of development. The grain was collected at the R8 stage of development (Gaska, 2006). The soluble, extractable Cry1Ac, Cry1F, and PAT proteins were measured using a validated enzyme-linked immunosorbent assay (ELISA). Cry1Ac, Cry1F, and PAT protein levels for all tissue types were calculated on a ng/mg dry weight basis. For Cry1Ac, average expression values ranged from 0.39 ng/mg dry weight in R3 stage root to 25.44 ng/mg dry weight in V5 stage leaf tissue (DAS 2012, Table 7, pg. 71). For Cry1F the average expression values ranged from 5.23 ng/mg dry weight in the R3 stage root to 56.75 ng/mg dry weight in the V5 stage leaf tissue (DAS 2012, Table 8, pg. 74). For PAT average expression values ranged from 0.63 ng/mg dry weight in the R3

stage root to 5.60 ng/mg dry weight in the V10-12 stage leaf tissue (DAS 2012, Table 9, pg.79). No Cry1Ac, Cry1F, or PAT protein was detected in the control (Maverick) tissues across the 10 locations.

Nutrition and Compositional Analysis was conducted and analyzed as evidence of no unintended effects to non-target organisms. The food and feed safety assessment of the Cry1Ac and Cry1F proteins expressed in DAS-81419-2 considers several factors, to include: safety of the donor organism, history of safe use, allergenic potential, toxicity potential, and dietary risk assessment based on consumption patterns. In 1997, EPA established an exemption from the requirement of a tolerance for the plant-incorporated protectant Cry1Ac in all plants (40 CFR part 174.510). Later EPA established an exemption from the requirement of a tolerance for the plant-incorporated protectant Cry1F in cotton (40 CFR part 174.504) and in corn (40 CFR part 174.520). The exemptions were based on safety assessments of the proteins including digestibility in simulated gastric fluid, lack of homology to known allergens and protein toxins, and lack of mammalian toxicity as demonstrated by acute oral mouse gavage studies. DAS has filed a petition with EPA for an exemption of a tolerance for Cry1F as expressed in soybean in 2012.

The Cry proteins expressed in DAS-81419-2 do not share any amino acid sequence similarities with known protein toxins which have adverse effects on mammals (FARRP, www.allergenonline.org, DAS-81419-2, pg. 80). Both Cry1Ac and Cry1F were rapidly digested in simulated gastric fluids in less than one minute indicating that the proteins are unlikely to elicit allergenic reactions when consumed and glycosylation analysis revealed no detectable covalently linked carbohydrates in Cry1Ac and Cry1F (DAS-81419-2, pg. 75). Additionally, neither protein caused adverse effects in mouse acute oral toxicity studies. Therefore, the low level Cry1Ac and Cry1F content in DAS-81419-2 relative to total plant proteins presents a low exposure risk to humans and animals (GenBank, www.ncbi.nlm.nih.gov/genebank), and the results of the overall safety assessment of the Cry1Ac and Cry1F proteins indicate that the proteins are not likely to cause allergic or toxic effects in humans or animals.

The PAT protein in DAS-81419-2 is 100% identical in amino acid sequence to PAT expressed in other transgenic crops that have been previously deregulated by USDA (USDA, 1996, 2001, 2004, 2005). The food and feed safety of PAT was assessed in these products and in published findings (OECD, 1999; Herouet et al., 2005) and shown to present no significant food or feed safety risk. The PAT protein is hydrolyzed rapidly in simulated gastric fluid and there was no evidence of acute toxicity in mice at a dose of 5000 mg/kg body weight of PAT protein (OECD, 1999). Additionally, updated bioinformatic analyses provided by the applicant revealed no meaningful homologies to known or putative allergens or toxins for their PAT amino acid sequence (FARRP, www.allergenonline.org, DAS-81419-2, pg. 80). Additionally, the EPA has concluded, after reviewing data on the acute toxicity and digestibility of the PAT protein, that there is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the PAT protein and the genetic material necessary for its production. The EPA has consequently established an exemption from tolerance requirements pursuant to Federal Food, Drug, and Cosmetic Act (FFDCA)

section 408(j)(3) for PAT and the genetic material necessary for its production in all plants (EPA, 1997).

There were no statistically significant compositional differences between the non-transgenic near-isogenic control (Maverick), reference variety entries, and DAS-81419-2 with regards to: 1) proximate, fiber, and mineral analysis of forage (DAS 2012, Table 11 and figure 33, pgs. 84-85), and 2) Mineral analysis of seed (DAS 2012, Table 13 and Figure 35, pgs. 90-91). There were three statistically significant findings with regards to proximate and fiber analysis of seed pertaining to fat, ash, and moisture. There was one statistically significant finding with regards to amino acid analysis of seed pertaining to phenylalanine. There were three statistically significant findings with regards to fatty acid analysis of seed that was measurable above the limit of quantitation pertaining to 16:0 palmitic, 18:3 linolenic, and 20:1 eicosenoic oils. There were two statistically significant findings with regards to vitamin analysis of seed pertaining to γ-tocopherol and Vitamin B5. There was one statistically significant finding with regards to bioactive analysis of seed which was total glycitein equivalent. However, all seven statistical differences between DAS-81419-2 and non-transgenic Maverick soybean fell within the accepted normal range compiled from six commercial reference varieties so the differences are not considered to be biologically meaningful. (DAS 2012, Tables 10-17, and Figures 33-39, pgs. 82-111).

Based on all the above noted considerations, APHIS concludes that Event DAS-81419-2 poses no more of a plant pest risk from new gene products, changes to plant metabolism or composition (with the exception of intended changes in insect resistance) than the near isogenic control soybean.

D. Potential Impacts on Disease and Pest Susceptibilities

APHIS assessed whether DAS-81419-2 is more likely than non-transgenic control soybean with regards to being more susceptible to diseases and pests. This assessment encompassed a thorough consideration of the introduced traits along with disease and pest susceptibility data from DAS-81419-2 field trials (DAS 2012, pgs. 113-120). Field tests were visually monitored for incidence of plant disease and pests on DAS-81419-2 and compared with the conventional soybean varieties, including Maverick and six non-transgenic reference lines at 10 sites located in: Richland, Iowa; Atlantic, Iowa; Carlyle, Illinois; Wyoming, Illinois; Frankfort, Indiana; Fisk, Missouri; La Plata, Missouri; York, Nebraska; Brunswick, Nebraska; and Germansville, Pennsylvania. Disease and insect damage was rated on a numerical scale of 0-100%, with 0% representing no damage due to disease incidence or insect pests. All plots were uniformly treated with pest control measures at each location based on local protocol. According to DAS, this design mimics commercial practice and allows comparison to typical cultivation conditions for non-transgenic soybean. There were no statistically significant differences between DAS-81419-2 and the non-transgenic near isogenic control Maverick in susceptibility to and interactions with diseases and insects (DAS 2012, Table 21, pg. 120). The data provided by DAS in the petition indicates that DAS-81419-2 is not biologically different from the non- transgenic control soybean with regards to increased pest susceptibility. Furthermore, the insect resistant and herbicide tolerant phenotype did not significantly

alter the non-target pest and disease incidences on DAS-81419-2 (DAS 2012, Section 8.2, pgs. 119-120); therefore, DAS-81419-2 is no more susceptible to pests and diseases compared to conventional soybean cultivars. In addition, the agronomic study evaluating plant growth characteristics throughout the growing season demonstrated the equivalence of DAS-81419-2 soybean with conventional non-transgenic soybean (DAS2012, Section 8.1 pgs. 113-118). Therefore, APHIS concludes that there is no indication that DAS-81419-2 will pose an increased plant pest risk.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

APHIS evaluated the potential for DAS-81419-2 to have damaging or toxic effects with regards to non-target organisms. Non-target organisms considered were representatives of the potentially exposed species in the expected southern U.S. agriculture environment. Estimated margins of exposure (MOE) (DAS 2012, pg. 127) were conducted on four non-target species: mouse, earthworm, collembola, and lady beetle for both Cry proteins (Cry1Ac and Cry1F). The MOE for all species tested was greater than ten times the no observed effect concentration (NOEC) (DAS 2012, pg. 128) with the exception of lady beetles and the Cry1Ac test. The potential risk was examined as the known spectrum of Cry1A is restricted to the order lepidoptera. An additional high-dose laboratory study of the Cry1Ac protein against lady beetles (Li et al., 2011) as well as data for coccinellids monitored in DAS-81419-2 field trials demonstrated no adverse effects on lady beetle performance and abundance (DAS 2012, pg. 128). A lack of adverse effects following exposure to Cry1Ac and/or Cry1F proteins has also been shown for collembolans (*Folsomia candida* and *Xenylla grisea*) (Sims and Martin, 1997), the minute pirate bug (*Orius albidipennis*) (González-Zamora et al., 2007), green lacewing (*Chrysopa carnea*) (Rodrigo-Simón et al., 2006), hymenopteran parasitoids (*Nasonia vitripennis*), honeybees (*Apis mellifera*), and bobwhite quail (*Colinus virginianus*). Agreement between laboratory and field observations for transgenic Bt proteins (Duan et al., 2009) has been supported through meta-analyses of NTO data at the order (Naranjo, 2009) and species-level (Duan et al., 2008). The meta-analysis for honeybees conducted by Duan included six studies incorporating Cry1Ac or Cry1F, suggesting that negative effects on pollinators is unlikely.

Because Cry receptors are not present in non-target birds and mammals, these insecticidal proteins are not expected to adversely affect non-target invertebrate and vertebrate organisms (Glare and O'Callaghan, 2000; EPA, 2008, Hofmann et al., 1988a; Hofmann et al., 1988b; Shimada et al., 2006a; Shimada et al., 2006b; Van Rie et al., 1989; Van Rie et al., 1990). The Cry proteins expressed in DAS-81419-2 poses little risk to aquatic organisms as the Cry1Ac and Cry1F proteins have been shown to have no adverse effects on fish or aquatic invertebrates due to the low likelihood of exposure and the lack of verified aquatic sensitive species (Carstens et al., 2011).

The potential impact on threatened and endangered species was assessed on four lepidopteran butterfly species that are known to co-occur with current soybean production sites. Ecology and life history information demonstrated that habitat requirements for larvae and adults of three of the identified species (Mitchell's satyr, Saint Francis' satyr, and Uncompahgre fritillary) do not overlap with commercial soybean

acreage, and are therefore not expected to be impacted by DAS-81419-2 cultivation (DAS 2012, pg. 130). There is a slight possibility of overlap for a fourth threatened species, the Karner blue butterfly. The potential for this species to be exposed to the Cry proteins is minimal as this lepidopteran larvae mainly feed on lupines (Montllor et al., 1990). Since soybean is not open-pollinated, the chance of the pollen getting on the leaves of the feeding source for the butterfly is negligible. There is no expected effect of the DAS-81419-2 on soil-dwelling organisms as Cry1Ac has been used extensively in sprayable formulations for over fifty years. Cry1F has been used as a sprayable formulation for over forty years (Schnepf et al., 1998; EPA, 1996). Laboratory experiments have shown that both Cry1Ac and Cry1F are quickly inactivated, and field studies have shown no accumulation of the proteins as a result of continuous planting of crops containing these proteins (Head et al., 2002; Herman et al., 2002; Shan et al., 2008). As discussed earlier in this section, neither of the proteins (PAT and Cry proteins), as expressed in this plant tissue are toxic or allergenic to non-target organisms.

F. Potential for Enhanced Weediness of DAS-81419-2 Soy

Soybeans are not considered a weed in the U.S. (Holm, 1977; Muenscher, 1980; Reed and Hughes, 1977), and are not listed as a Federal noxious weed (7 CFR part 360). APHIS assessed whether DAS-81419-2 is any more likely to become a weed than the non-transgenic comparator, or other cultivated soybeans. The assessment encompassed a thorough consideration of the basic biology of soybeans and an evaluation of the unique characteristics of DAS-81419-2. APHIS analyzed the field test reports and other data included in the petition, as well as data from scientific literature on the establishment, reproduction, and dispersal processes of soybeans as measures of invasiveness and weediness of Cry1Ac, Cry1F, and PAT expressing soybean. Agronomic data including; early population, seedling vigor, days to flowering, disease incidence, insect damage, days to maturity, lodging, plant height, final population, number of pods, number of seeds, shattering, yield, and weight per 100 seeds was researched in the field trials for DAS-81419-2. Agronomic properties of DAS-81419-2 related to weediness, such as emergence (DAS 2012, pgs. 113-118), seedling vigor (DAS 2012, pgs. 113-118), response to environmental stressors (DAS 2012, pgs. 119-120), and germination (DAS 2012, pgs. 121-122), have been shown to be equivalent to conventional soybean. There were only two characteristics with respect to emergence and seedling vigor (days to flowering, and number of pods) that showed up outside the statistical significance of the Maverick variety, however, they were within the range for the non-transgenic reference varieties used as a control group (DAS 2012, pg. 118). The results of the 13 evaluated plant growth and development characteristics showed the introduced traits did not unexpectedly alter the phenotypic or agronomic characteristics of DAS-81419-2 compared to conventional soybean (DAS 2012, Table 19, pgs. 117-118).

The germination and dormancy evaluation for DAS-81419-2 was performed under both cold and warm conditions. Germination of DAS-81419-2 did not differ significantly from that of the non-transgenic near-isogenic control Maverick under cold ($P = 0.5372$) and warm ($P = 0.6129$) conditions. The results indicate that seed dormancy characteristics have not been significantly changed in DAS-81419-2 (DAS 2012, Table 22, pg122). Since seed dormancy is a contributing factor as to whether a plant will become an

invasive weed, it is important to consider how possible changes will affect the environment. The higher the dormancy the higher chance the seed will exist to propagate into unwanted plants during a later growing season (Martinez-Ghersa et al., 2000). This data confirms that DAS-81419-2 dormancy has not been significantly changed from the non-transgenic near-isogenic control Maverick and is therefore unlikely to persist as a weed.

As a whole, DAS-81419-2 does not exhibit a statistically significant decrease in fitness when compared to the Maverick control and reference varieties which could be indicators of increased plant pest and insect predation. The results on growth characteristics, seed production, and germination indicate that the DAS-81419-2 is not significantly different than its comparators. There is no indication that DAS-81419-2 possesses a selective advantage that would result in increased weediness. Therefore, DAS-81419-2 lacks the ability to persist as a troublesome weed (Baker, 1965), and there would be no direct impact on current weed management practices for soybean cultivation. Even if soybean volunteers were to establish, there are effective weed management strategies to control such volunteers (Beam et al., 2005; OECD, 2000; York, 2005).

G. Potential Impacts on the Weediness of Any Other Plants with which It Can Interbreed

In assessing the risk of gene introgression from DAS-81419-2 to its sexually compatible relatives APHIS considers two primary issues: 1) the potential for gene flow and introgression, and 2) the potential impact of introgression.

The potential for vertical gene flow from DAS-81419-2 into its sexually-compatible relatives is negligible in the U.S, Brazil, and Argentina as wild soybean species do not exist naturally in the North American continent (OECD, 2000). Soybean is separated into three categories; wild (*Glycine soja*), weedy (*Glycine gracilis*), and cultivated (*Glycine max*). These species grow wild or semi-wild in Asia. Fertile hybrids between *G. max and G. soja* (Nakayama and Yamaguchi, 2002; Mizuguti, 2010; Ohigashi et al., 2010), and between *G. max* and *G. gracilis* occur (Karasawa, 1952). *Glycine soja* and *Glycine gracilis* grow naturally only in Asia, not in the United States (Lu, 2005). The subgenus *Glycine* consists of twelve wild perennial species. These species grow wild in Australia, South Pacific Islands and Asia (Newell and Hymowitz, 1978), and do not exist naturally in the U.S.

Soybean is predominantly a self-pollinated species (OECD 2000), yet a small amount of outcrossing does occur. Soybean typically exhibits a level of cross-pollination below one percent. Adjacent rows are measured to have between 0.03 and 3.62% outcrossing, but plants more than 4.5 meters apart cross at less than 0.02% (Caviness, 1966; Yoshimura, 2006; Matsuo et al., 2006). While most sources agree that insects do not greatly increase outcrossing rate in domestic soybean (Erickson, 1984), there is some evidence that some insects such as honeybees can increase hybridization rates (Free, 1970; McGregor, 1976). Pollen is only viable for two to four hours as it desiccates quickly with pollen shed normally occurring late in the morning (Caviness, 1966). Current cultivation practices to prevent out-crossing have been deemed sufficient to prevent unwanted gene flow. For

soybean, the Association of Official Seed Certifying Agencies (AOSCA) requires that "Fields of soybeans shall be separated from any other variety or uncertified seed of the same variety by a distance adequate to prevent mechanical mixture".

Cultivated soybean has never been found in the wild without human intervention (Hymowitz and Singh, 1987). Therefore, it is highly unlikely that soybean plants in the U.S.will be found outside of an agricultural setting. It is also highly unlikely that gene flow and introgression will occur between DAS-81419-2 and soybean plants in a natural environment (feral soybean) as cultivated soybean in the U.S. are self-pollinating with no pollen vector. Soybean seeds have negligible dormancy characteristics which allow them to germinate and become volunteers only in the same field in the following growing cycle, and only if field conditions are still optimal for seed germination. Even if this soybean could exist outside agriculture as a volunteer plant, it lacks the ability to persist as a troublesome weed, and there would be no direct impact on current weed management practices for soybean cultivation. USDA has therefore determined that any adverse consequences of gene flow from DAS-81419-2 to wild or weedy species in the United States are highly unlikely.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS considered whether there are likely to be significant changes to agricultural practices associated with cultivation of DAS-81419-2; and if so, are they likely to significantly exacerbate plant diseases or pests, especially those for which APHIS has a control program. There will be no change to current agricultural practices with the introduction of DAS-81419-2. The efficacy study demonstrates that Cry1Ac and Cry1F expressed in DAS-81419-2 provide protection against target lepidopteran pests only. Currently Bt cotton and corn is rotated on fields with both Bt and non-Bt soybean since the granting of non-regulated status to Monsanto 87701 Insect Resistant Soybeans in 2010 (09-082-01p). The addition of a DAS-81419-2 slightly increases the chance that Bt plants will be grown all season long in areas where there used to be a break with the cultivation of non-Bt soybean. Planting of DAS-81419-2 for breeding and seed increase purposes only in the U.S. is not likely to have any effect on insect resistance as the level of selection pressure on other lepidopteran pests of soybean will be negligible under the proposed acreage limits. This petition will be administered under a FIFRA section 3 seed increase permit only within the U.S. and will fall within the guidelines of established Bt refuges set by the EPA (DAS 2012, pg.139). DAS-81419-2 does not differ from conventional soybean in agronomic characteristics (DAS 2012, Section 8, pgs. 113-122) and thus is not expected to impact U.S. soybean grain or seed production.

DAS-81419-2 expresses the PAT protein and therefore it is tolerant to the herbicide glufosinate. Glufosinate tolerance in soybeans is currently available today to U.S. growers; therefore, no impact is expected from the deregulation of DAS-81419-2 on current weed management practices.

The data presented in this petition demonstrates that there are no biologically meaningful differences between DAS-81419-2 and conventional, non-transgenic soybean in agronomic characteristics (DAS 2012, Section 8, pgs.113-122). There are no new phenotypic characteristics in DAS-81419-2 to indicate it is any different from conventional soybean with regards to weediness potential, and like conventional soybean, the risk of gene flow from DAS-81419-2 to wild relatives in the U.S. is negligible. No significant impact is expected on current crop management or crop rotation practices that would inhibit the granting of non-regulated status to DAS-81419-2 soybean.

I. Potential Impacts from Transfer of Genetic Information to Organism with which DAS 80419-2 Soy Cannot Interbreed

APHIS assessed the likelihood of transfer of transgenes from DAS-81419-2 into other organisms with which soybean does not interbreed. To date, there has been no documented definitive example of horizontal gene transfer between plants and microbes (Conner et al., 2003). There are many opportunities for plants to directly interact with common environmental microorganisms such as fungi and bacteria, (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores), and so far there are no reports of significant horizontal gene transfer between sexually incompatible or evolutionarily distant organisms (Keese, 2008).

The bacteria with close associations to plants such as *Agrobacterium* and *Rhizobium* have been sequenced and currently there is no evidence that these organisms contain genes derived from plants (Kaneko et al., 2000; Kaneko et al., 2002; Wood et al., 2001). Where review of documents such as Brown's infer that horizontal gene transfer may have occurred; the transfer is believed to have occurred over millions of years (Brown, 2003; Koonin et al., 2001; Yoshida, Maruyama et al., 2010). It is important to state that the genes and promoter sequences for use in this soybean event are optimized for plant expression; not bacterial expression, so even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. The FDA has evaluated horizontal gene transfer from the use of antibiotic resistance marker genes, and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (FDA 1998); furthermore, DAS-81419-2 contains no antibiotic resistant transgene proteins.

APHIS concludes that horizontal gene transfer is unlikely to occur from Event DAS-81419-2 to microorganisms and thus no significant plant pest risk is expected from horizontal gene transfer.

J. Conclusion

APHIS has reviewed the information submitted by the petitioner and conducted a plant pest risk assessment on DAS-81419-2 compared to the unmodified near isogenic soybean variety from which it was derived and several non-transgenic commercial varieties. Based on information on the biology of soybean (OECD, 2000), data presented by DAS in the petition (DAS 2012), and scientific data relevant to a discussion of plant pest risk,

APHIS concluded the following regarding DAS-81419-2: Due to the lack of plant pest risk from the inserted genetic material, the lack of weediness characteristic, the lack of atypical responses to disease or plant pests in the field, the lack of deleterious effects on non-targets or beneficial organisms in the agro-ecosystem, lack of plant pest effects from changes in agricultural/cultivation practices, the lack of horizontal gene flow, or potential to impact the weediness of other plants with which it can interbreed, APHIS concludes that DAS-81419-2 is unlikely to pose a plant pest risk.

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